SYNOPSIS

Of The Research Work Leading to The Degree of

Doctor of Philosophy

In Botany

Entitled

"TISSUE CULTURE AND PHARMACOLOGICAL STUDIES ON *ECHINOPS ECHINATUS* ROXB. AND *MERREMIA RHYNCORHIZA* HALLIER"

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2022

1. Introduction:

World Health Organization (WHO) has estimated that 80% population of the developing countries relies on traditional **medicines** for their primary health care. India is home to a variety of traditional medicine systems that support many of the health care needs of this country's nearly 1 billion people. In India, there are almost 17,000 species of the flowering plants, of which about 8,000 species are claimed to have medicinal properties (Anonymous, 2000).

The fact that derivatives of medicinal plants are non-narcotic, have no ill effects and constitute the natural base for treatments, have resulted in an increase in demand of these plants in developing and developed countries. There is growing demand for natural products based medicines and other things due to which destructive harvesting of plants is being carried out (Tripathi, 2008).

With the prevalent deforestation of the planets, many medical, fascinating and rare plants are destroyed before scientists determine their all medicinal values and states in phylogenic history or resemblance in the process of evolution.

There is increase in demand of natural products in national and international market. Therefore, almost 90% of collection of medicinal plants is done from the wild, which includes destructive harvesting. Due to this, many species have become endemic and endangered. Therefore, the task force committee set up by Planning Commission, Govt. of India has recommended that it is necessary to conserve and to cultivate medicinal plants for sustainable use by using *in vitro* propagation techniques. Also, it is recommended to isolate active compounds and produce phytochemicals for formulation of drugs for various uses (Anonymous, 2003).

It is also necessary to find out bioactivities of wild plants, so that new sources of remedies for different diseases/ disorders are found out.

***Merremia rhyncorhiza* Hallier:** There are about 40 species of *Merremia* distributed in

warmer parts of the world. In India, it is found in Western Ghats of Maharashtra and adjoining parts of Cannara of Karnataka (Cooke, 1908). *Merremia rhyncorhiza* of Convolvulaceae is an endangered, endemic, perennial and spreading hirsute herb. It bears a bunch of robust tuberous roots. Locally, tuberous roots known as Ran ratale or Nangarvel in Marathi, are consumed raw/cooked mainly by cowherds. This is apparently the main reason for this taxon to have reached endangered status (Surwase, 2000). It is reported to be rare. The tubers are eaten and greedily sought by the natives. The leaves are also used as vegetable (Cooke, 1908). Watve (2013) reported this species as endemic to Maharashtra. Each plant bears a bunch of 15 nepiform tubers. Short aerial erect stem bears prostate spreading branches. Leaves tri lobed, inflorescences are axillary cymes with yellow flowers. Flowering and fruiting occurs in October.

Merremia rhyncorhiza is used as food because tubers are eaten and greedily sought by the natives. The leaves are also used as vegetable (Cooke, 1908). *Merremia rhyncorhiza* is reported as one of the endemic and endangered species which is not studied much. Tubers are used on stomatch ache.

***Echinops echinatus* Roxb.** It is commonly known as Usnakantaka and there are about 120 species of the genus distributed in Europe, Asia and Africa. It is an erect, rigid, pubescent, annual herb about 1 m in height. It has short, stout stems with branches widely spreading from the base. The leaves are alternately arranged, sessile, oblong, deeply pinnatifid,7-12 cm long, and covered with cottonywool beneath, the lobes are triangular, simuate, and prickly, and the spines are often 2.5 cm long. Flower heads occur in solitary white spherical balls (globose), 3-5 cm across, clustered at the ends of branches.

The plant is recommended in jaundice, hysteria, dyspepsia, hoarseness of throat and cough. The powdered root is used as antilice and also applied to wounds of cattle for killing maggots (Murad, 2011).

The plant is alterative, analgesic, antifungal, anti-inflammatory, contraceptive, diuretic, febrifuge, hepatic, nervine, ophthalmic and stomachic.

It is used in the treatment of hoarse coughs, hysteria, dyspepsia, scrofula and various eye problems. It is also used to treat brain disease, chronic fever, pains in the joints, inflammations.

The root is abortifacient, aphrodisiac.

The powdered roots are applied to wounds in order to destroy maggots and, combined with the gum from Acacia trees, is applied to the hair to destroy lice.

The dried and powdered root bark is mixed with milk (100g/250ml) and used as a treatment for diabetes.

Mixed with water to make a paste, the root bark powder is applied to the male genitals one hour before intercourse in order to treat poor sexual vitality.

The fumes obtained from burning the leaves and roots is inhaled as a treatment of respiratory disorders, especially asthma, in order to obtain rapid and long-lasting relief.

The seeds are sweet and aphrodisiac.

The plant is known to contain various medicinally active compounds including the polyphenol apigenin; flavanone glycosides with known anti-inflammatory activities; and various alkaloids (Pirjade *et al.,* 2015 and Maurya *et al.,* 2015 *).*

2. Review of Literature and Development in the Subject (Previous work done in the relevant subject):

Agrawal, Nahata and Dixit (2012) studied the protective effects of *Echinops echinatus* on testosterone-induced prostatic hyperplasia in rats. They conducted studies to assess the 5α-reductase inhibitory potential of *E. echinatus*. This was followed by *in vivo* studies on the potent extracts. Testosterone (3 mg kg^{-1}, s.c.) was administered to the rats along with the test extracts (50 and 100 mg kg^{-1}, p.o.) for a period of 28 days.

Chaudhuri (1987) reported a new alkaloid from *Echinops echinatus* by using spectroscopic data analysis. In addition to echinopsine and echinopsidine, a new alkaloid, echinozolinone, has been identified in *Echinops echinatus* as 3(2-hydroxyethyl)-4(3H)-quinazolinone from its spectral data.

Eram *et al.* (2013) reported that the aerial plant parts of *Echinops echinatus* could act as an effective hepatoprotective material. For this purpose, the aerial plant parts were subjected to *in vivo* hepatoprotective study in order to validate its traditional use in hepatobiliary disorders, by native people of Cholistan Desert, Pakistan. Hepatoprotective effects of pre-treatment with aerial parts (ethanolic extract) of *E. echinatus* (EE), (500 and 750 mg/kg/day, p.o., for 7 days) against CCl4 (0.75 ml/kg., s/c.) intoxicated rabbits were evaluated by serum biochemical parameters and liver histopathological observations. EE Extract (500 mg/kg) produced more significant results as compared to extract (750 mg/kg). However, (both doses of 500 and 750 mg/kg) showed hepatoprotection as obvious by significant reinstatement of levels of SGOT, SGPT, ALP and even TB as compared to CCl4 and Silymarin control groups.

Fatima *et al.* (2017) reported anti-diabetic activity of hydro-alcoholic root extract of *Echinops echinatus* and its beneficial effects on nephropathy in experimental rats. They studied anti-diabetic activity of 70% Hydro-alcoholic root extract of *Echinops echinatus* at a dose of (100& 200 mg/kg body weight p.o) was investigated in diabetes Nephropathic rats. α- Amylase inhibitory activity of the *Echinops echinatus* was determined against Diastase and has shown 72% inhibitory effect of amylase at conc of 25 mg/ml. When treated with 70% Hydro-alcoholic root extract of *Echinops echinatus* at a dose of 100 and 200 mg/kg b.w p.o. Group IV and group V has produced significant regeneration of cells in diabetic nephropathic rats under histopathological studies. The effect was more pronounced in 200 mg/kg b.w than 100mg/kg body weight & showed Nephroprotective as obvious by significant reinstatement levels of Oral glucose tolerance test, blood glucose levels in biological parameters serum urea, serum creatinine &serum lipid profile (Triglycerides, Total cholesterol, HDL, LDL, VLDL).

Jamila *et al.*, (2020) reported elemental analysis and bioactivities of *Echinops echinatus*. They quantify 28 mineral elements total phenolic and flavonoid contents, antioxidant, and anti-cholinesterase activities in its leaves, stem, flowers and achene extracts. In elemental analysis, the concentrations of Ca (9199.3 to 63975.6 µg/g) and K (7866.1 to 42700.6 µg/g) were comparatively high. They also reported ethyl acetate

extract was the most enriched extract with phenolic and flavonoid contents as well as the most potent free radical scavenger (DPPH IC50 9.54 μg/mL, ABTS IC50 5.88 μg/mL). In anti-diabetic activity, leaves and stem methanol extract was the strong inhibitor of α-glucosidase enzyme with IC50 values of 371.4 and 368.6 μg/mL, respectively. Methanol and ethyl acetate extracts also showed dual cholinesterase enzymes inhibition.

Kamila *et al.*, (2018) reported antihemolytic activity of *Echinops spinosus*. They studied that the medicinal plants possess therapeutic properties. In this study, tannin extracts of the leaves, stems, and roots of *Echinops spinosu*s which belong to the Asteraceae plant family of Tlemcen (north of Algeria) are screened for the antihemolytic activity toward human erythrocytes. The good results of the antioxidant activity of *E. spinosus* prompted us to lead a reflection on their antihemolytic activity.

Kapoor and Lakhera (2013) reported the ethnomedicinal plants of Jodhpur District, Rajasthan. They discussed about Ethnomedicinal uses of *Echinops echinatus*. For pregnancy, the infusion of leaves and inflorescence is given to the barren lady early in the morning for seven days in the Kathodi tribes. They also put inflorescence on fire and inhale fumes through mouth to cure teeth infection. Garasia tribe in Jodhpur district uses decoction of roots which is placed in the navel of women for easy and quick delivery.

Maurya and Seth (2015) reported ethnomedicinal review of *Echinops echinatus*. The roots, leaves, fruit, and bark are extensively used in folk medicine as well as in Ayurveda. Also, the plant shows a wide range of pharmacological activities such as antifungal, analgesic, diuretic, reproductive, hepatoprotective, antioxidant, anti-inflammatory, wound-healing, antipyretic, and antibacterial properties. Among the several active constituents, apigenin, apigenin-7-O-glucoside, echinaticin, 5,7-dihydroxy-8,4'-dimethoxy-flavanone-5-O-α-L-rhamnopyranosyl-7-O - β-D-arabinopyranosyl-(1→4)-O-β-D-glucopyranoside are the most important in terms of reported pharmacological activities.

Othaman *et al.,* (2022) reported antidiabetic properties of *Echinops spinosus.* To investigate the anti-diabetic properties of both ES total extract (ESTE) and its high flvonoids fraction (ESHFF) in experimental diabetes induced by streptozotocin (STZ) injection in rats. Forty-two rats were divided into six groups. Diabetes was induced using STZ (55 mg/kg). Seven days after STZ administration, the diabetic animals were treated daily with ESTE, ESHFF, or metformin (MET) as a standard anti-diabetic drug for 28 days. Blood and tissues samples were collected for biochemical, molecular, and histological investigations. Both ESTE and ESHFF demonstrated anti-diabetic properties, as evidenced by lowering glucose levels and increasing the levels of insulin, insulin receptor expression rate, and glycogen synthesis.

Rafay *et al.,* (2021) reported the phytochemical and antimicrobial activities of *Echinops echinatus*. *Echinops echinatus* was evaluated for phytochemicals and antibacterial activities against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* and Anti-urease inhibition assay activities were tested during the study. The leaves showed the maximum inhibition zone 10mm against *K. pneumonia* while against *E. coli, S. aureus* and *P. aeruginosa* was found inactive. *K. pneumoniae, E. coli* and *P. aeruginosa* were resistant to flowers extract. The root extract showed maximum zone of inhibition 18 mm against *S. aureus* while against *P. aeruginosa* and *K. pneumonia* inhibition zone was 15mm and 14mm respectively. The extracts of leaves, flowers, roots and stem showed positive results for αglucosidase. The roots extract showed maximum inhibition with 75.3±1.5 with IC50 value of 207.3±1.3 for α-glucosidase. Urease inhibitory activity of stem extracts showed maximum inhibition activity 93.71±0.86 with IC50 value 15.63±0.42. While leaves and roots showed almost same activity 92.45±0.63 and 92.63±0.76, respectively.

Singh et al., (1988) gave a report on antifungal activity of new flavones and flavones glycosides of *Echinops spinosu*s. They isolated two new compounds namely echinacin permethyl ether and apigenin-5,4′-dimethyl ether which had antifungal activity against *Alternaria tenuissima* (Kunz. ex Pers.) Wiltshire. *Alternaria tenuissima* causes leaf blight disease in pigeon pea. These phenolic

compounds apigenin, apigenin-7-O-glucoside, echinacin, and echinaticin showed high efficacy against the pathogen at concentrations ranging from 25 to 150 μg mL−1. Echinacin, which was highly effective at 150 μg mL−1, is considered the most promising of these compounds and its use as a control measure against *Alternaria* blight of pigeon pea under field conditions has been suggested.

Singh *et al.,* (1989) reported anti-inflammatory activity of *Echinops echinatus*. They conducted experimental studies to prove anti-inflammatory activity on ethanol extract of *Echinops echinatus* whole plant. The extract effectively inhibited the acute inflammation induced in rats by carrageenan, formaldehyde and adjuvant and the chronic arthritis induced by formaldehyde and adjuvant. The extract was more effective parenterally than orally. The toxicity studies showed reasonable safety warranting further studies.

Syedikramsharif (2018) reported the toxicity and antiulcer activity of ethanolic extract of *Echinops echinatus*. They concluded that the preliminary phytochemical evaluation of Ethanolic extract of *Echinops echinatus* Roxb. From the results of present study it was concluded that the ethanolic extract of *Echinops echinatus Roxb* (whole plant) at 400mg/kg shows significant anti-ulcer activity in animals models used, as compared to the standard drug Omeprazole. The positive anti-oxidant activities in different methods further confirm its protection against gastro duodenal pathogenesis.

Tungenwar *et al.,* (2020) reported the standardization of nursery technology in two endemic and endangered species. They studied the nursery techniques such as sprouting response of horizontally cut upper 1/4th tuber segments was similar to that of whole tubers in both the selected species. Also, seed germination obtained with needle scarification gave 80% germination within 8-10 days for both the species. Half strength MS alone was the best for in vitro seed germination with 70 % result for *Flemingia tuberosa a*nd 80 % result for *Merremia rhyncorhiza* species respectively.

3. Objective of Research:

By considering the above importance, the present work would be undertaken with the following objectives:

1) To standardize micropropagation protocol of the selected species through direct clonal propagation and through induction of callus.
2) To standardize regeneration protocol of the selected species through somatic embryogenesis by using different explants.
3) To acclimatize *in vitro* regenerated plantlets of the selected species.
4) To standardize production and optimization of secondary metabolites through callus, normal root and hairy root culture etc. by using different explants.
5) Pharmacological studies of natural plants and *in vitro* produced secondary metabolites of the selected species.

4. Methodology to be adopted:

Material and Methods

1) Micropropagation through callus: - Different explants like leaf discs, nodal or apical tips etc. would be used in different suitable media with different concentrations of cytokinins and auxins for callusing, shoot and root generation.

2) Direct regeneration of shoots from vegetative explants: - Direct regeneration from young leaf discs explants or other explants would tried in suitable medium with different concentrations of cytokinins and auxins.

4) Rooting in *in vitro* generated shoots: - Shoots obtained from nodal, apical, leaf discs, cotyledonary explants, and other all tried explants shall be subjected to rooting in medium containing different concentrations of different auxins

5) Acclimatization of plantlets: - The plantlets formed will be transferred either into vermiculite and then into pots or into vermiculite- soil or sand-soil mixture and then grown in the shade house. These, plantlets would be transferred to natural habitats as a conservation strategy.

6) Somatic emryogenesis: - Different explants would be used for callusing and to develop somatic embryos. Later, these somatic embryos would be germinated in suitable medium.

7) Secondary metabolite production:-

Callus culture, cell suspension culture, root culture by using shake flask culture would be utilized for production of secondary metabolites.

Optimization of secondary metabolites would be done by-

1) Manipulation of media components, phytoharmones, pH, temperature, agitation, light effect etc.

2) Exogenous supply of biosynthetic precursors to culture medium.

3) Use of biotic and abiotic elicitators etc.

4) Hairy root culture would be produced by using *Agrobacterium rhizogenes* strains.

8). **Pharmacological Activity:-** Pharmacological studies like antimicrobial / antifungal activities would be done by using disc plate method and food poisoning techniques respectively.

For anti diabetic property alloxan diabetic Wistar rats would be used. For anti-inflammatory activity paw volume in Carrageenin injected wistar rats will be used. Other

5. Importance of study / Society application:

C. Significance of the study:

1. The present investigation would develop the protocol of rapid production of planting material for plantation, which is required for sustainable use of this species.
2. The study would add to the information about in vitro production of secondary metabolites for the commercial use.
3. It would also help to promote the cultivation of the selected species so that, this species would not become endemic and endangered.

4. It would also reveal various pharmacological properties like antimicrobial, antidiabetic, anti-inflammatory and hepatoprotective activities of the selected plant species.

6. Proposed work plan / Formulation and Structure of Study:

Program of research work

Ist Year:

1) Collection of wild plant material like tubers and seeds of the selected species.
2) Conventional nursery technology with respect to seed germination, effect of soil texture and FYM supplement on seedling health and growth.
3) Micropropagation through callus.

II^{ed} Year:

4) Direct regeneration from other vegetative explants.
5) Somatic embryogenesis.
6) In *vitro* rooting of generated shoots.
7) Acclimatization of produced plantlets and transfer in nature for conservation.

IIIrd Year:

8) Production and optimization of secondary metabolites in callus culture, cell suspension culture, organ culture (normal roots) and hairy root culture.
9) Biological activities of the selected plant species

Suryawanshi G A — (Signature of Student)

Dr B S Surwase — (Signature of Guide)

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